

WHAT IS CLAIMED IS:

- sub C17
1. A cell of interest producing the donor substrate CMP-SA above endogenous levels. C
 2. A cell of interest producing an acceptor substrate, the donor substrate MP-SA, and expressing the enzyme sialyltransferase; wherein said acceptor substrate is a glycan.
 3. The cell of claim 2 wherein said glycan is a branched glycan comprising GalGlcNAcMan by at least one branch of said glycan and said Gal is a terminal Gal.
 4. The cell of claim 3 wherein said glycan is an asparagine-linked glycan.
 5. A cell of interest producing sialylated glycoprotein above endogenous levels. D
 6. The cell of claim 5, wherein said glycoprotein is asparagine (N)-linked.
 7. The cell of claim 5, wherein said glycoprotein is heterologous.
 8. The cell of claim 7, wherein said heterologous glycoprotein is mammalian.
 9. The cell of claim 5, wherein said mammalian glycoprotein is selected from the group consisting of plasminogen, transferrin, Na⁺, K⁺-ATPase, and thyrotropin. C
 10. The cell of claim 5, wherein said cell expresses at least one enzyme selected from the group consisting of:
 - a) GlcNAc-2 epimerase;

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sub C2
cont.

- b) an enzyme catalyzing conversion of UDP-GlcNAc to ManNAc;
- c) sialic acid synthetase;
- d) aldolase;
- e) CMP-SA synthetase;
- f) CMP-SA transporter; and

wherein said expression is above endogenous levels.

11. The cell of claim 10, wherein said cell expresses enzyme (a).

12. The cell of claim 11, wherein said enzyme is human.

13. The cell of claim 10, wherein said cell expresses enzyme (b).

14. The cell of claim 13, wherein said enzyme is human.

15. The cell of claim 10, wherein said cell expresses enzyme (c).

16. The cell of claim 15, wherein said cell expresses the enzyme of SEQ

ID NO:6.

17. The cell of claim 10, wherein said cell expresses enzyme (d).

18. The cell of claim 17, wherein said cell expresses the enzyme of SEQ

ID NO:2.

19. The cell of claim 10, wherein said cell expresses enzyme (e).

20. The cell of claim 19, wherein said cell expresses the enzyme of SEQ

ID NO:4.

21. The cell of claim 10, wherein said cell expresses enzyme (f).

22. The cell of claim 21, wherein said enzyme is human.

sub C3 } 23. The cell of claim 10 wherein said cell further expresses at least one enzyme selected from the group consisting of:

- a) Gal T;
- b) GlcNAc TI;
- c) GlcNAc TII;
- d) sialyltransferase; and

wherein said expression is above endogenous levels.

24. The cell of claim 10, wherein activity of endogenous N-acetylglucosaminidase is suppressed.

25. A kit for expression of sialylated glycoproteins, comprising the cell of claim 1.

26. A method for manipulating glycoprotein production in an insect cell, said method comprising enhancing expression of at least one enzyme selected from the group consisting of:

- a) GlcNAc-2 epimerase;
- b) an enzyme catalyzing conversion of UDP-GlcNAc to ManNAc;
- c) sialic acid synthetase;
- d) aldolase;
- e) CMP-SA synthetase;
- f) CMP-SA transporter; and

wherein the expression of each enzyme expressed is enhanced to above endogenous levels.

27. The method of claim 26, wherein expression of enzyme (a) is enhanced.

28. The method of claim 27, wherein said enzyme is human.

29. The method of claim 26, wherein expression of enzyme (b) is enhanced.
30. The method of claim 29, wherein said enzyme is human.
31. The method of claim 26, wherein expression of enzyme (c) is enhanced.
32. The method of claim 31, wherein said enzyme has the sequence of SEQ ID NO:6.
33. The method of claim 26, wherein expression of enzyme (d) is enhanced.
34. The method of claim 33, wherein said enzyme has the sequence of SEQ ID NO:2.
35. The method of claim 26, wherein expression of enzyme (e) is enhanced.
36. The method of claim 33, wherein said enzyme has the sequence of SEQ ID NO:4.
37. The method of claim 26, wherein expression of enzyme (f) is enhanced.
38. The method of claim 35, wherein said enzyme is human.
39. The method of claim 26, further comprising enhancing expression of at least one enzyme selected from the group consisting of:
- a) Gal T;

- b) GlcNAc TI;
- c) GlcNAc TII;
- d) sialyltransferase; and

wherein the expression of each enzyme expressed is enhanced to above endogenous levels.

sub C 40. The method of 26, further comprising suppressing activity of endogenous N-acetylglucosaminidase.

41. A method for producing sialylated glycoproteins, said method comprising expressing a heterologous protein in an insect cell manipulated according to the method of claim 26.

42. The method of claim 41, wherein said heterologous protein is mammalian.

43. The method of claim 42, wherein said mammalian protein is selected from the group plasminogen, transferrin, Na^+ , K^+ -ATPase, thyrotropin.

44. A method for producing a sialylated glycoprotein in a cell of interest said method comprising:

- a) determining the carbohydrate substrates in said cell;
- b) transforming said cell with enzymes to produce necessary precursor substrates; and
- c) constructing a processing pathway in said cell to produce a sialylated glycoprotein.

45. The method of claim 44 wherein said cell is selected from the group consisting of yeast, insect, fungal, plant, and bacterial cells.

46. The cell of claim 10, wherein said cell expresses both enzyme (c) and enzyme (e).

47. ¹⁰ The method of claim 26, wherein expression of both enzyme (c) and enzyme (e) is enhanced.

Add C

Add B

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